Research Project Report

BCG VACCINATION IN SILICOSIS

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by

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Saranac Lake, New York

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~~Final~~ report 12-31-53 USN
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Discussion and Summary

The present report gives the results of a series of experimental studies begun in 1948 and aided by a contract between the Office of Naval Research, Department of the Navy and the Saranac Laboratory of the Trudeau Foundation; previous contract number N7onr-307 Task II NR 131-211 and current number NR 105-002. These studies pertain to vaccines derived from particular BCG strains under cultivation in the Tice Laboratory and relate to the original study conducted under this contract.

(Discussion and Summary continued on following page)

~~Final~~ report 12-31-53 USN
N7onr-307 NR 131-211 NR 105-002

BCG VACCINATION IN SILICOSIS

This report pertains to experimental studies concerning the course of infection with attenuated BCG strains of tubercle bacilli in tissue under the influence of free crystalline silica. The studies are extensions of previous investigations which have been conducted by the Saranac Laboratory, some under contract with the Biological Sciences Division, Office of Naval Research, Department of the Navy, Washington 25, D. C.

The previous investigations have been reported elsewhere (1, 2, 3). The original report concerned studies employing the BCG strain 826L supplied by Dr. S . R. Rosenthal, Medical Director, Research Foundation, Tice Laboratory, Chicago, Illinois. The summary of that report is as follows:

1. The BCG organisms spread from an intracutaneous site of inoculation in guinea pigs and may localize in the the viscera, including the lung and the tracheobronchial lymph nodes.

2. The resistance of the host can be lowered by the tissue reaction to quartz particles so that the BCG organisms in the dose used for vaccination or for infection by inhalation can produce progressive and fatal pulmonary disease.

3. BCG organisms recovered from tissue exhibiting progressive disease have not been altered by growth in silicotic tissue for they show no inhancement of virulence when subinoculated subcutaneously in large doses (10 mg) into healthy guinea pigs.

4. Vaccination with BCG organisms does not prevent or retard the development of tuberculosilicosis produced by an infection with the attenuated R1 strain of the human-type tubercle bacillus in the presence of inhaled quartz dust.

Subsequent to the unexpected and striking observation that inhaled quartz dust alters the tissue susceptibility to attenuated BCG organisms, the Saranac Laboratory initiated a number of additional investigations, which are related, but each of which has rather well-defined objectives. One of those investigations (2) was supported by funds from the American Trudeau Society and by a James Alexander Miller Fellowship award. That investigation concerned a series of experimental studies to determine whether quartz dust would also have an adverse effect upon infection with any of three other strains of acid fast organisms, namely: the attenuated bovine tubercle bacillus, BCG strain Phipps 873 obtained from Dr. J. D . Aronson; the avirulent mycobacterium tuberculosis variant, H37Ra strain; and the nonpathogenic bacillus, Mycobacterium marinum strain. Since those studies are relevant to the current report for the Office of Naval Research, it seems pertinent to include here a few statements summarizing certain of the findings derived from those studies. The statements are these :

The experimental investigations substantiate previous studies concerning the BCG strain Tice 826L and demonstrate that:

1. Tubercle bacilli of the attenuated BCG strain Phipps 873 and of the avirulent human strain H37Ra may disseminate from an intracutaneous site of inoculation. The BCG organisms may seed the lung and the tracheobronchial lymph nodes of normal and of silicotic guinea pigs. The H37Ra organisms may seed the tracheobronchial lymph nodes of silicotic pigs, but those organisms were not isolated from the nodes of the normal pigs or from the lungs of the normal or of the silicotic pigs. In contrast, bacilli of the nonpathogenic M. marinum strain remained localized at the intracutaneous site of inoculation and were not isolated from the lungs or nodes of any of the animals.

2. Tubercle bacilli of the attenuated BCG strain, Phipps 873, may produce progressive and often fatal tuberculosis as a complication of the silicotic process in the lungs and tracheobronchial lymph nodes of guinea pigs with prolonged exposure by inhalation to crystalline free silica in the form of quartz dust. Tubercle bacilli of the avirulent H37Ra strain and of the nonpathogenic M. marinum strain fail to accomplish such effects.

3. Tubercle bacilli isolated from the lungs and from the tracheobronchial lymph nodes of both the silicotic and the nonsilicotic guinea pigs retained the cultural characteristics of the organism (the attenuated BCG strain Phipps 873 and the avirulent strain H37Ra) originally injected intracutaneously into those pigs; and those bacilli exhibited no enhancement of virulence when subinoculated subcutaneously in large doses (10 mg) into healthy guinea pigs.

4. The mechanism whereby crystalline free silica in the form of quartz dust deposited in tissue accomplishes its adverse effect upon a complicating tuberculous infection in silicotic guinea pigs appears to be a function of lowered tissue immunity rather than of direct action of the silica upon the infecting tubercle bacillus.

5. It should be emphasized that the observations noted were derived from experimental investigations with animals and that the findings apply only to the influence of inhaled quartz dust upon a tuberculous infection produced by BCG organisms in guinea pigs.

Other investigations (3) were supported by the Office of Naval Research and are the major subject of this report. They were designed in order to verify the results of previous studies (1) concerning the Tice strain of BCG organisms, to establish the manner in which crystalline free silica exerts its adverse effect upon a tuberculous infection, and to determine the length of the post vaccination period during which this adverse effect can occur.

Experimental Protocol

The series of investigations comprising this report may be considered from the point of view of a number of separate, but related experiments, identified as follows:

Experiments 1 and 2(Saranac Laboratory numbers 1133 and 1160): The influence of inhaled silica upon infection produced in guinea pigs and dogs by intracutaneous BCG vaccines (Tice strains 885L, 890L, 907L) prepared in the Tice Laboratory and forwarded by air mail to the Saranac Laboratory.

Experiments 2, 3, and 4 (Saranac Laboratory numbers 1160, 1150 and 1154): The influence of silica upon infection produced in dogs, rabbits and guinea pigs by intracutaneous BCG vaccines (Tice strains 626L and 924L) prepared in the Saranac Laboratory.

Experiment 5 (Saranac Laboratory number 1189): The influence of inhaled silica upon infection produced in guinea pigs by intracutaneous vaccine, fresh and four days old respectively (Tice strain 924L) prepared in the Saranac Laboratory.

Experiment 6 (Saranac Laboratory number 1153): The pulmonary reaction of guinea pigs to inhaled silica and to an inhaled suspension of dead BCG organisms (Tice strain 826L) in a vaccine prepared in the Saranac Laboratory.

Experiment 7 (Saranac Laboratory number 1200): The influence of inhaled silica upon infection produced in dogs by intracutaneous BCG vaccine (Tice strain 924L) prepared in the Saranac Laboratory.

Experiment 8 (Saranac Laboratory number 1226): The influence of daily parenteral cortisone (cortone acetate) upon infection in guinea pigs produced by intracutaneous BCG vaccine (Tice strain 926L) prepared in the Saranac Laboratory.

Experimental Materials and Methods

BCG organisms: The BCG strains were obtained originally from the culture depot of Tice Laboratory, Chicago, Illinois. The strains were forwarded to the Saranac Laboratory where they were maintained on glycerolated-water-potato medium, on ATS medium, or on both mediums. Later subcultures were made on liquid Sauton's medium to produce a surface film of bacterial growth for the vaccines which were prepared by the Saranac Laboratory. The various strains employed were the 826L, used in the original experiment (1), and its daughter strains 885L, 890L , 907L and 924L.

Vaccines: The vaccines used in the various experiments identified previously have a very direct relationship in that each one contained the organisms of a cultured strain of BCG which was derived genetically from the BCG strain 826L. The vaccines differed, however, in their preparation and subsequent handling.

In the instance of experiment 1 and for part of experiment 2, the vaccines, three in number, were prepared in the Tice Laboratory. These vaccines, suspended in fluid and contained in small vials, were shipped in thermos bottles by air mail from Chicago and were received from 2 to 4 days later in Saranac Lake. Upon receipt, the respective vaccines were diluted ~~in~~ with one part Sauton's medium and three parts of 0.85 per cent sodium chloride. Sufficient diluent was added to make a final concentration which would permit the desired dose of a vaccine for each animal. Ziehl-Neelsen smears made from each

vaccine revealed many acid fast organisms as single bacterial units; however, a number of small clumps were also present.

In all the other experiments (part of experiment 2 and experiments 3 to 8 inclusive) the vaccines were prepared in The Saranac Laboratory from cultures as received from the Tice Laboratory or as maintained subsequently on glycerolated-water-potato medium, on ATS medium or on both mediums. In each instance, the respective culture was subcultured on glycerolated-water-potato medium. At the end of two weeks incubation, the film growth on the fluid portion of the medium was transferred to several flasks of Sauton's medium. After twelve days incubation, the surface film was removed and placed between sterile pads of blotting paper to remove excess moisture. The dried film was weighed and then triturated lightly in a sterile mortar with pestle in a diluent consisting of one part Sauton's medium and three parts of 0.85 per cent sodium chloride. Sufficient diluent was then added to make the desired final concentration of each vaccine.

The vaccine employed in experiment 6 was prepared as described in the preceding section. In addition, in order to obtain as uniform a suspension as possible for inhalation, the vaccine was centrifuged for 6 minutes at 1500 r.p.m. The resultant supernatant, collected in a vial, was heated for one hour in a water bath at 60 degrees centigrade. The concentration of killed bacilli was such that there were approximately 100 singly dispersed acid fast bacilli in an oil immersion field of a smear one square centimeter in area produced

by a sample delivered from a loop 3mm in diameter. The injection of 10 mg subcutaneously into a series of healthy guinea pigs produced only small local lesions without disease in the draining lymph nodes or elsewhere in other tissues of the body.

Viability of vaccines: In the case of experiments 1, 2 and 5, the respective vaccines were studied for viability of bacilli. Each vaccine was diluted with 0.1 per cent bovine albumin in distilled water. Separate drops of the diluent, usually 10^{-6}, 10^{-7} and 10^{-8}, were inoculated on to the surface of a number of plates of oleic-acid albumin-agar medium (4) using the techniques described by Fenner et al (5). The extent of growth was determined by reading the numbers of viable bacterial units (colony counts) at intervals of 2, 4 and 6 weeks.

Vaccination: In all experiments except number 4 (1154), the intracutaneous vaccinations were accomplished by injecting into each animal a volume of the suspension containing 0.1 mg of the respective BCG strain of organisms. The animals in experiment 4 received 0.015 mg of the organisms. The injection was made into the skin of the shaved flank.

In experiment 3 (1150), the vaccine was injected into the ear vein of rabbits, each of which received a dose consisting of 1 cc containing 0.1 mg of the BCG organisms .

For the inhalation experiment, using killed organisms, the vaccine was placed in a DeVilbiss atomizer. Each animal was exposed to a spray

produced by twelve puffs from the atomizer and delivered during a period of one minute. Complete details for the inhalation method routinely employed by this laboratory have been described elsewhere (6).

Dust exposure: The methods for exposing animals to quartz dust by inhalation have been described in detail in other publications (7). Briefly, the technique consisted of exposing the animals to a cloud of the fine quartz particles disseminated in the air of a room by mechanical devices designed to produce the desired dispersion and concentration of particles in the atmosphere. Samples of the atmospheric dust were collected from time to time with a Greenburg-Smith impinger apparatus. Study of these samples by the light field microscope technique (8 and 9) revealed that the atmospheric concentration of the dust was approximately 500 million particles per cubic foot of air. An analysis of the size distribution of the particles showed that more than 99 per cent were less than 10 microns in diameter. Chemical analysis showed a total silica content greater than 99 per cent. The animals in all the inhalation experiments of this report were exposed to the dust for approximately eight hours daily, 5½ days a week. At other times they lived in normal air.

Cortisone: Saline suspension of "cortone" acetate, Merck. Each guinea pig received daily 4 mg injected subcutaneously into the groin or the axilla.

Animals: The guinea pigs were from the colony bred and reared in the Trudeau Animal House. The rabbits and dogs were purchased in the open market.

Experimental Results

Experiment 1 (1133): Inhalation of quartz dust and intracutaneous BCG vaccine in guinea pigs.

The studies comprising this experiment were presented in detail in the previous progress report (3), and therefore only brief mention will be made here of certain data.

The two vaccines, from BCG strains 835L and 890L, were prepared by the Tice Laboratory and were received approximately three days later in Saranac Lake. Viability counts of each vaccine revealed 380 million units of BCG organisms per ml of the 835L vaccine and 370 million units per ml for the 890L vaccine.

The data summarizing the results are presented in table 1 of this current report. Suffice it to state here that the data demonstrate that the BCG organisms of strains 835L and 890L migrate from an intracutaneous site of inoculation in the guinea pig and that some of those organisms localize in the viscera, including the lungs and the tracheobronchial lymph nodes. In that respect these results agree with those in the original experiments (1) in which the mother BCG strain 826L was employed. However, the results do not completely agree with the original findings since the occurrence of detectable progressive tuberculosis was much less frequent and less advanced in the silicotic animals and since there were relatively fewer deaths from tuberculosilicosis. These differences were disturbing

and therefore a series of new experiments were initiated in an attempt to discover the possible reasons for failure in agreement between the experiments.

Experiment 2 (1160): Inhalation of quartz dust and intracutaneous BCG vaccination in dogs.

This experiment was conducted not only to extend the original study (1), but more specifically it was designed to determine, by the use of long lived animals such as the dog, the length of the post vaccination period during which a pulmonary infection resulting from an intracutaneous localization of BCG organisms would be subject to the adverse influence of inhaled quartz dust.

The disconcerting results, with the guinea pigs in experiment 1, directed attention to the group of dogs which had received the vaccine prepared by the Tice Laboratory from BCG strain 907L, a daughter strain of 826L. Three silicotic dogs, 6, 9, and 8, table 2, had been killed; one at 365 days and two at 395 days after vaccination. They failed to show any evidence of spreading tuberculosis as a complicating feature of the silicotic process present in their lungs and tracheobronchial lymph nodes.

In view of those negative findings, and because of the possibility that the organisms comprising the particular vaccine used may have lost much of their ability to multiply in silicotic tissue, the surviving dogs, 1 to 5 inclusive, and living in normal air, were revaccinated.

This vaccine was prepared in the Saranac Laboratory and was derived from the BCG strain 924L, also a descendant of strain 826L, but culturally maintained in the Saranac Laboratory. Viability counts of this vaccine revealed 210,000,000 units of organisms per ml. Each dog received intracutaneously 0.5 ml of the vaccine, equivalent to 0.1 mg of BCG organisms . Subsequently three of the dogs, number 3, 4 and 5, were kept in the dust room in which quartz dust was disseminated; and the other two dogs , 1 and 2, were kept in normal air to serve as control animals to the BCG vaccination.

In summary, all dogs developed lesions at the site of the intracutaneous localization of the vaccine. The lesions produced by the first vaccination developed slowly and by 21 days had progressed to maximum induration, which was accompanied by ulceration in seven of the eight dogs. Subsequently, the lesion in each of the dogs began to heal and by 42 days it exhibited gross retraction with the formation of scar tissue. At that time and during the succeeding 15 days, all dogs were tested with old tuberculin. The tests were negative to 0.0003 cc of 3 per cent, 0.003 of 3 per cent and 0.015 cc of 15 per cent, but strongly positive to 0.1 cc of 100 per cent tuberculin.

The cutaneous lesions produced by the second dose of vaccine developed rapidly with definite induration at 9 days, ulceration in three dogs at 14 days and in another at 20 days. The lesion in one dog failed to ulcerate. By 55 days the lesions in all dogs had regressed to scar formation.

Tuberculosis has not been discovered in the organs, including the lungs and tracheobronchial lymph nodes, of any of the dogs thus far killed at periods as long as 940 days post vaccination and 545 days with inhalation exposure to quartz dust. The remaining two dogs of this experiment will be killed at a later date. It is anticipated, however, that the results in them will not be significantly different from those already observed.

Thus, this experiment fails to meet the designed objective, because spreading tuberculosis did not develop in the silicotic dogs. In retrospect, these negative results with dogs might have been anticipated in view of the subsequent findings in guinea pigs vaccinated with the same strain of BCG organisms.

Experiment 3 (1150): Intravenous injection of quartz dust followed by intravenous inoculation of BCG organisms into rabbits.

This experiment constituted part of the studies of the previous report (3) and is introduced here for completeness of this report. The object of this experiment was to determine whether quartz particles injected intravenously and localized in the spleen, liver, hepatic lymph node and lungs would stimulate in those tissues a progressive tuberculosis initiated by the intravenous inoculation of a definite dose of BCG organisms .

The vaccine was prepared in the Saranac Laboratory from a culture of BCG organisms, Tice strain 826L. Four rabbits were injected twice weekly for 10 weeks with 5 cc of a 0.5 per cent suspension of quartz

particles 1 to 3 microns in size. Thus, each rabbit received a total dose of 0.5 grams of quartz dust. These four rabbits and four additional infection control rabbits were then inoculated intravenously with an amount of vaccine containing 0.1 mg of the BCG organisms . One animal from each group was killed at 30, 60 and 120 days after infection and the surviving animal in each group was to be killed 365 days after infection. However, since the last animal in the group exposed to dust died at 210 days, the experiment was terminated at that time by killing the last infection control rabbit.

The results of this experiment are summarized in table 3. It is noted that there was marked increase of tuberculous disease in the hepatic lymph nodes in all of the rabbits in the dust-exposed group as compared with the amount of disease in the hepatic lymph nodes of the infection control group. This was not unexpected because it is a common observation that following the intravenous injection of quartz dust a large amount of that dust is quickly deposited in the hepatic lymph node.

The livers of the rabbits of both groups showed no difference in the amount of tuberculous disease. In the spleens of the rabbits of the dust-exposed group it was impossible to differentiate microscopically whether the minute collections of macrophages present in the tissue were stimulated by the quartz dust or by the BCG organism. This difficulty arises from the fact that in the rabbit collections of macrophages respond to the stimulation of quartz dust and also

to the stimulation of the tubercle bacillus. Detection of acid-fast organisms in such collections would have aided in the interpretation of the disease in that organ. In the lungs, for the first two months, there was a definite increase in the number and size of the tubercles in the rabbits receiving quartz dust, as compared with the infection control animals. Subsequently, the pulmonary tuberculosis resolved in the lungs of the rabbits of both groups. In the kidneys there was no evidence that the quartz dust had unduly stimulated the tuberculous disease, which was no more severe than in the kidneys of the infection control animals. Organisms were isolated from the tissues, as noted in table 11.

This experiment partially substantiates the original observations that the localization of BCG organisms in tissue under the influence of free crystalline silica may eventuate in the development of tuberculous disease. However, the results, fail to fully corroborate the previous observations since the tuberculous disease which occurred was less extensive and less progressive than anticipated.

Experiment 4 (1154): The inhalation of quartz dust and intracutaneous vaccination with a small dose of BCG organisms in guinea pigs.

The object of this experiment was to determine whether a vaccinating dose smaller than that used heretofore could liberate sufficient numbers of viable organisms into the lung and tracheo-bronchial lymph nodes which under the influence of silica would develop subsequently a spreading tuberculo-silicosis. For that

purpose a series of guinea pigs were inoculated intracutaneously with 0.015 mg of vaccine prepared in the Saranac Laboratory from the BCG strain 826L of the original experiment (1) and grown in the Saranac Laboratory.

The results of this experiment are summarized in table 4. It will be observed that none of the pigs developed spreading tuberculosis, irrespective of whether they were of the silicotic group or of the control group living in normal air. The small dose of BCG vaccine used may be a factor responsible for the failure of this experiment to verify the original study in which a dose approximately seven times greater was used.

Experiment 5 (1189): Inhalation of quartz dust and intracutaneous vaccine, fresh and four days old, of the BCG strain 924L in guinea pigs.

As stated previously, the results obtained in the original experiment (1) were not completely verified by the results of experiment 1 of this report. It will be recalled that both experiments utilized vaccines of BCG organisms, the former experiment strain 826L and the latter experiment the daughter strains 885L and 890L. In attempting to determine the reasons for the lack of verification, the experiments were scrutinized for possible differences in techniques which may have been responsible. It was recognized that in comparison with the BCG strain used in the original experiment, the daughter strains may have become further attenuated, if not avirulent, during the course of the sub-culturing prior to preparation of the vaccine,

and thus may have lost their capacity to produce progressive tuberculosis in silicotic tissue. The experiments differed, however, in another seemingly more important respect, namely: in the original study a vaccine was used which was freshly prepared in the Saranac Laboratory and immediately injected into the animals; in the second study, experiment 1 of this report, the vaccines were prepared in the Tice Laboratory and flown by air to Saranac Lake. However, approximately four days elapsed between the preparation and the use of the Tice vaccines. It was thought that the elapsed time may have lessened the viability of the organisms contained in those vaccines and thus may have accounted for the observed failures. Consequently, experiment 5 was initiated to determine whether the age of the vaccines may have been responsible.

The vaccine employed in experiment 5 was prepared in the Saranac Laboratory from the Tice strain 924L, also a daughter strain of the original 826L. The strain 924L was being maintained on cultures in the Saranac Laboratory. Immediately after preparation, the fresh vaccine was tested for viability. A measured amount of that vaccine, containing 1 mg of organisms per ml, was mixed with 0.1 per cent bovine albumin in distilled water until 10^{-6}, 10^{-7}, and 10^{-8} dilutions were obtained. Aliquots of each dilution, measuring 0.02 ml, were inoculated on to the surface of plates of oleic-acid albumin-agar medium of Dubos (4). The plates were incubated and read at 2, 4 and 6 weeks. The average reading of the plates revealed 210,000,000 units of organisms per ml. Immediately after preparation the fresh vaccine was injected in a series of guinea pigs each of which received intra-

cutaneously a volume of 0.1 ml containing 0.1 mg of BCG organisms . At that time, a portion of the vaccine was placed in the refrigerator at 38 degrees Farenheit where it was kept for four days. After refrigeration, tests for viability revealed an average of 172,000,000 units of organisms per ml. Immediately after refrigeration, the four day old vaccine was injected intracutaneously into a series of pigs, each pig received a volume of 0.1 ml containing 0.1 mg of BCG organisms .

With respect to the inhalation of quartz dust, the experiment involved three groups of guinea pigs, namely:. a group which had 120 days of dust exposure prior to vaccination; a second group in which the exposure to dust and the vaccination were initiated simultaneously; and a third group which had no exposure to the quartz dust and which served as the infection control animals.

The detailed results of the experiment are recorded in tables 5 to 10 inclusive. The pertinent data is summarized for comparative purposes in tables 11 and 12. It will be noted that following vaccination, all animals manifested positive skin reactions to intracutaneous old tuberculin. The tuberculin reactions in the various animals ranged from 1^+ to 4^+ on the first test and in most animals had declined on other tests during the subsequent months. In addition, the frequency of 3^+ and 4^+ reactions was less pronounced in the silicotic pigs than in the non-silicotic infection control animals. Irrespective of these reactions, however, there was no significant difference in results observed in the various

groups of animals. A positive culture was obtained from the lung or from the tracheobronchial lymph node of a very occasional animal. Only one of the silicotic animals, #35 killed at 182 days after vaccination, tables 6 and 11, revealed an isolated non-spreading tubercle in a tracheobronchial lymph node. Other pigs, whether silicotic or non-silicotic, remained free of detectable organ tuberculosis following their vaccination. Thus, there was no demonstrable difference in the infectability of the fresh vaccine as contrasted with that of the four day old vaccine prepared from the same BCG strain of organisms. It is important to note here that the organisms subcultured from the tissues of some of the animals were tested for virulence by injecting 10 mg subcutaneously into a series of healthy guinea pigs. The pigs which were killed for study at intervals up to 240 days after injection have shown no evidence of tuberculosis .

Although this experiment also failed to verify the original observations (1), it demonstrated that in the instance of these experiments the age (not older than 4 days) of the vaccine was not a factor responsible for the failure.

Experiment 6 (1153): Inhalation of quartz dust and inhalation of a vaccine of dead BCG organisms in guinea pigs.

During the course of the experimental studies concerning inhaled quartz dust and its effect upon infection produced by BCG organisms, it became important to know the extent to which a silicotic process in the lung would be modified by the deposition of small numbers of

dead BCG organisms such as may be disseminated from an intracutaneous site of vaccination. Although similar studies had been carried out heretofore with other strains of tubercle bacilli, experiments had never been conducted with strains of BCG organisms . Therefore, a group of fourteen guinea pigs which had been exposed for three months to the inhalation of quartz dust were exposed by inhalation, for 1 minute as previously described, to a spray of BCG organisms derived from strain 826L under cultivation in the Saranac Laboratory and killed by heating. The animals were immediately returned to the dust room where their exposure to quartz dust was continued. In addition, another group of ten pigs was similarly exposed to the atomized spray of the same vaccine. These pigs were kept in normal air and served as the BCG infection control animals.

Animals from each group were killed for study at 90, 180, 270, 365 and 540 days after exposure to the vaccine. The guinea pigs exposed to the inhalation of quartz dust exhibited a progressive development of inflammatory reaction characteristic of the tissue response to free crystalline silica. Initially, the reaction consisted of scattered focal accumulations of macrophages in the alveolar spaces with slight histiocytic proliferation which thickened the surrounding alveolar walls. Ultimately, the lung and the tracheobronchial lymph nodes revealed scattered fibrotic lesions with pronounced hyalinization. There was no evident modification of the silicotic reaction by the dead BCG organisms which may have been deposited in the lung.

The non-silicotic BCG control animals revealed initially only a very rare microscopic collection of macrophages in an occasional alveolar space. Some of those collections may have been due to localization of BCG organisms; however, they could not be differentiated from similar collections found occasionally in the lungs of healthy guinea pigs. The tracheobronchial lymph nodes in all pigs remained normal.

In view of these results, it is obvious that the progressive tuberculous disease with cavitation which occurred in many of the silicotic guinea pigs in the original and the subsequent experiments (1 and 2) could not have been due to dead BCG organisms.

Experiment 7 (1200): Intracutaneous vaccination with a freshly prepared BCG vaccine in dogs whose exposure to dust was initiated at different periods of time after vaccination.

This experiment was undertaken concurrently with and as an extension of experiment 2 in which dogs were also employed and in which the experimental results had failed ultimately to confirm previous observations (1) concerning the adverse effect of inhaled silica upon infection with BCG organisms, Tice strains 826L. Furthermore, this experiment also had the objective to determine the length of time during which the BCG organisms disseminated from the intracutaneous site of vaccination to the lung and to the tracheobronchial lymph nodes would remain sufficiently viable to be influenced by free crystalline silica deposited in those tissues.

Consequently, each of six dogs was vaccinated intracutaneously with 0.5 ml of a freshly prepared vaccine containing 0.1 mg of BCG organisms of the Tice strain 924L which was under cultivation in the Saranac Laboratory and which was used also in the experiment 2 employing dogs. The inhalation exposure to quartz dust was started at different intervals after vaccination, namely, immediately in two dogs, approximately seven months later in one dog and 12 months after vaccination in another dog. Two dogs have been kept in normal air.

The protocol of this experiment is summarized in table 13. It will be noted that animal number 5 died seventeen days after vaccination: the cause of death remains undetermined. Another animal, number 6, was killed for study at 540 days after vaccination. Its lungs and tracheobronchial lymph nodes disclosed a moderately advanced silicosis of the simple type. There was no gross evidence of complicating tuberculosis .

The experiment is a current one and will be reported upon at a later date. In retrospect, however, it is anticipated that the results of this experiment will not be significantly different from those in experiments 2 and 5 in which dogs and guinea pigs vaccinated with the same BCG strain of organisms also failed to verify the original experiment (1).

Experiment 8 (1226): Intracutaneous BCG vaccination and daily subcutaneous cortisone in guinea pigs.

The object of this experiment was to learn whether substances other than free crystalline silica would also have an adverse influence upon BCG vaccination and upon organisms which may disseminate from the intracutaneous site to distant tissues .

Since adrenocorticotrophic hormone (ACTH) and cortisone exert a profound effect on many inflammatory reactions, both infectious and non-infectious, it was expected that the course of infection due to BCG organisms would be modified by these compounds. Consequently, an experimental study was initiated which involves the following groups of guinea pigs:

GROUP I - Vaccination superimposed upon cortisone:

Daily cortisone started 2/13/53

Vaccination, 0.1 mg BCG, 2/27/53

Daily cortisone continued, 2/27/53

Cortisone discontinued, 12/26/53 25 pigs

GROUP II - Cortisone superimposed upon vaccination:

Vaccination, 0.1 mg BCG, 2/27/53

Daily cortisone started, 5/27/53

Cortisone discontinued, 12/26/53 25 pigs

GROUP III - Vaccination control:

Vaccination, 0.1 mg BCG, 2/27/53 — 25 pigs

GROUP IV - Virulence test:

Vaccination, 10 mg BCG, 2/27/53 — 25 pigs

The vaccine was prepared in the Saranac Laboratory from a culture of BCG organisms, Tice strain 826L, which had been maintained in the laboratory.

The cortisone is a saline suspension of "cortone" acetate (Merck). Each daily dose consists of 4 mg injected subcutaneously into the groin or the axilla.

The experimental study is currently underway, but certain pertinent observations may be made at this time, table 14. Animals of Groups I, II and III have been skin tested with 0.1 cc of 3 per cent old tuberculin at intervals after vaccination. With reference to those reactions it will be noted that there is considerable variation in the intensity of reaction manifested by the animals comprising each group. In general there is no consistent significant difference in sensitivity to tuberculin at the various rest periods. This was expected. More significant, however, is the apparent fewer number of animals with 3^+ and 4^+ reactions to the test at 18 weeks and subsequently in the cortisone groups than in the non-cortisone-BCG control animals of Group III. Apparently the cortisone inhibits the development of the inflammation reaction of the skin to tuberculin.

As of the date of this report animals of Groups I, II and III have been killed at 120, 240 and 300 days following vaccination. They fail to show gross evidence of organ tuberculosis. During the course of the succeeding months, additional animals will be sacrificed at intervals for purposes of study.

The animals in Group IV, which had been injected with 10 mg of the vaccine for test of virulence, have been killed at 30, 60, 120 and 240 days after vaccination. None of those animals reveal evidence of gross organ tuberculosis. Thus, it is apparent that the BCG organisms, Tice strain 626L, during artificial cultivation did not acquire an enhanced virulence for normal guinea pigs.

In summary, this experiment is currently underway, and subsequent observations to be made on the remaining animals will disclose whether daily cortisone administered subcutaneously has an effect upon infection occasioned by BCG organisms injected intracutaneously.

Discussion and Summary

The present report gives the results of a series of experimental studies begun in 1948 and aided by a contract between the Office of Naval Research, Department of the Navy and the Saranac Laboratory of the Trudeau Foundation; previous contract number N7onr-307 Task II NR 131-211 and current number NR 105-002. These studies pertain to vaccines derived from particular BCG strains under cultivation in the Tice Laboratory and relate to the original study conducted under this contract.

The studies have been conducted concurrently or in sequence with other studies supported by a grant from the Committee on Medical Research of the American Trudeau Society, Medical Section of the National Tuberculosis Association, and with the help of a James Alexander Miller Fellowship award. These other studies concern respectively vaccines from a Phipps strain of BCG, and H37Ra strain and the M. marinum strain of organisms.

In the main, the overall objective of the combined studies is an attempt to establish the mechanisms whereby free crystalline silica exerts its adverse influence upon tissue infected with tubercle bacilli. More specifically, the objective is pointed primarily to experimental infection produced by vaccines containing BCG organisms. Within the framework of that specific objective it should be mentioned here that BCG vaccine is defined as a suspension of viable tubercle bacilli derived from a bovine strain which has been attenuated to the point where it is unable to produce progressive disease. This definition is a matter of conjecture. There exists some fear that the bacilli might revert to ~~its~~ their original virulent state or that unfavorable circumstances in the host may lower the tissue immunity to a degree enabling the attenuated bacilli to produce a progressive tuberculous disease. The existence of such circumstances in the silicotic animal has been demonstrated by the Saranac Laboratory in studies involving at least two vaccines of two different BCG strains of organisms. The results with those vaccines were similar in that the intracutaneous localization of each had produced progressive

tuberculosis in the lungs and tracheobronchial lymph nodes of silicotic pigs. However, in the instance of one of those strains, Tice 626L, vaccination with a daughter strain had failed to verify the original results. Consequently, a series of experiments were conducted in order to determine the factors responsible for that failure. These experiments indicate that the vaccines prepared from subcultures of that strain and from its daughter strains contain organisms which:

1. Are viable as demonstrated by artificial culture on suitable mediums.
2. Produce significant lesions at the site of intracutaneous localization in amounts of 0.1 mg.
3. Sensitize the host to intracutaneous test doses of old tuberculin.
4. Disseminate occasionally from the intracutaneous site of vaccination to distant organs.

In general, however, the experiments have failed to verify the original study concerning the ability of that particular vaccine to produce progressive tuberculosis in tissue under the influence of quartz. The question immediately arises as to whether or not that vaccine was contaminated with another strain of more virulent bacilli. This does not appear to be the case, however, since the organisms recovered from the tuberculo-silicotic tissue in animals of that study did not produce progressive tuberculosis when sub-injected in large doses (10 mg) into non-silicotic pigs and since another experiment employing a different vaccine, Phipps BCG strain, corroborated the original study. Therefore, it appears that:

1. The Tice BCG strain 826L and its daughter strains employed in these experiments are unstable.

2. Those strains under condition of artificial culture undergo continued attenuation even to the point of being incapable of producing progressive organ tuberculosis in silicotic guinea pigs. In that respect those strains act as the avirulent Ra dissociate of the H37 strain of tubercle bacilli employed in other experiments.

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TABLE 1

INHALATION OF QUARTZ DUST AND INTRACUTANEOUS BCG VACCINATION
(TICE STRAINS 885L and 890L) IN GUINEA PIGS
(Experiment 1 - #1133)

Summary of Results

Group	Duration of Observation	Number of Animals Observed	Animals Killed			Animals Died				Cultures
						Of Pneumonia, etc.				
			Definite Pulmonary Tuberculosis	No Pulmonary Tuberculosis	Tracheobronchial Lymph Node Tuberculosis	Of Pulmonary Tuberculosis	Definite Pulmonary Tuberculosis	No Pulmonary Tuberculosis	Tracheobronchial Lymph Node Tuberculosis	Organisms Isolated*
1: Intracutaneous BCG (Tice strain 885L) and Inhalation of Quartz Dust Simultaneously	months 22	25	4	10	3	0	3	8	5	0
2: Intracutaneous BCG (Tice strain 885L) with Inhalation of Quartz Dust Started at 3 Months After Inoculation	21	25	0	15	1	0	0	9	1	0
3: Intracutaneous BCG (Tice strain 885L) with Inhalation of Quartz Dust Started 6 Months After Inoculation	22	25	0	19	0	0	0	6	0	1
4: Intracutaneous BCG (Tice strain 885L) with Inhalation of Quartz Dust Started 12 Months After Inoculation	22	25	0	21	0	0	0	4	0	0

*Number of positive cultures from each group of animals

TABLE 1 continued on next page

TABLE 1 (Con't)

Group	Duration of Observation	Number of Animals Observed	Animals Killed			Animals Died				Cultures
							Of Pneumonia, etc.			
			Definite Pulmonary Tuberculosis	No pulmonary Tuberculosis	Tracheobronchial Lymph Node Tuberculosis	Of Pulmonary Tuberculosis	Definite Pulmonary Tuberculosis	No Pulmonary Tuberculosis	Tracheobronchial Lymph Node Tuberculosis	Organisms Isolated*
5: Intracutaneous BCG (Tice strain 885L); Infection Control Animals for Groups 1, 2, 3, 4	months 22	65	0	63	0	0	0	2	0	0
6: Inhalation of Quartz Dust for 6-1/2 Months, Then Intracutaneous BCG (Tice strain 890L), Then Continuation of the Exposure to Quartz Dust	19-1/2	20	6	9	5	0	1	4	2	3
7: Inhalation of Quartz Dust for 6-1/2 Months, Then Intracutaneous BCG (Tice strain 890L), and Removal of Animals to Normal Air	19-1/2	20	2	16	6	0	0	2	0	5
8: Intracutaneous BCG (Tice strain 890L); Infection Control Animals for Groups 6 and 7	19-1/2	20	0	20	0	0	0	0	0	2

*Number of positive cultures from each group of animals

TABLE 2

INHALATION OF QUARTZ DUST AND INTRACUTANEOUS BCG VACCINATION
(TICE STRAINS 907 L AND 924L) IN DOGS
(Experiment 2 - #1160)

Dog Number	Vaccination BCG 907L	Dust Exposure	Revaccination BCG 924L	Dust Exposure	Total Days Vaccinated	Total Days Dust Exposure	Pathology
	days	days	days	days	days	days	
7	365	365	0	0	365	365	Simple silicosis. No spreading tuberculosis.
8	395	395	0	0	395	395	Simple silicosis. No tuberculosis.
6	395	210	0	0	395	210	Simple silicosis. No tuberculosis.
3	395	0	365	365	760	365	Silicosis. No tuberculosis.
4	395	0	545	545	940	545	Silicosis. No tuberculosis.
5*	395	0	545 +	545 +	940 +	545 +	
control 2	395	0	365	0	760	0	No tuberculosis.
control 1*	395	0	365 +	0	760 +	0	

*Dogs #1 and #5 are still living.

TABLE 3

INTRAVENOUS INJECTION OF QUARTZ DUST FOLLOWED BY INTRAVENOUS INOCULATION OF BCG ORGANISMS (TICE STRAIN 326L) INTO RABBITS
(Experiment 3 - #1150)

Animal Number	Quartz Dust	BCG Vaccine	Mode of Death	Pathology				
				Lung	Liver	Hepatic Lymph Node	Spleen	Kidney
	days	days						
3	30	30	K	IS-*	NS -*	CO-*	?-*	O-*
1	60	60	K	IS-P	NS-P	IS-*	?-P	NS-*
2	120	120	K	O-O	O-P	IS-*	?-O	NS-*
5	210	210	D	O-O	O-O	IS-*	O-O	O-O
7	0	30	K	NS-*	NS-*	NS-*	O-*	#-*
6	0	60	K	NS-P	NS-O	O-*	NS-P	NS-*
8	0	120	K	O-O	O-P	?-*	O-O	#-*
9	0	210	K	O-O	O-*	?-*	O-O	IS-P

Key

K Killed for sampling
D Died
O No recognizable tubercles; no positive culture
NS Recognizable non-spreading tubercles
IS Isolated spreading tubercles
CO Isolated spreading tubercles with coalescence
P Positive cultures
No histology
* No bacteriology
? Small collection of macrophages, possibly due to localization of tubercle bacilli but difficult to differentiate from similar collection of macrophages responding to quartz dust

TABLE 4

INHALATION OF QUARTZ DUST AND INTRACUTANEOUS BCG VACCINATION WITH 0.015 mg BCG ORGANISMS (TICE STRAIN 326L) IN GUINEA PIGS
(Experiment 4 - #1154)

Animal Number	Quartz Dust	BCG Infection	Pathology
	days	days	
2	180	180	Immature silicosis. No tuberculosis.
3	180	180	Immature silicosis. No tuberculosis.
5	365	365	Mature silicosis. No tuberculosis.
6	365	365	Mature silicosis. No tuberculosis.
7	540	540	Mature silicosis. No tuberculosis.
8	540	540	Mature silicosis. No tuberculosis.
11	0	180	
12	0	180	
13	0	365	
14	0	365	
15	0	540	No tuberculosis observed in the
16	0	600	lungs, tracheobronchial lymph nodes,
17	0	600	spleen, liver, hepatic lymph nodes,
18	0	600	kidneys.
19	0	600	
20	0	600	

TABLE 5

INHALATION OF QUARTZ DUST FOR 120 DAYS,
THEN INTRACUTANEOUS BCG VACCINATION (FRESHLY-PREPARED VACCINE TICE 924L)
WITH INHALATION OF QUARTZ DUST CONTINUED
(Experiment 5 - #1139)

Guinea Pig Number	Date of Infection	Tuberculin Skin Test				Mode of Death	Time after Infection	Tuberculosis		Organisms Isolated	
		3-3-52	5-14-52	9-16-52	1-14-53			Lungs	Tracheo-bronchial Lymph Nodes	Lungs	Tracheo-bronchial Lymph Nodes
							days				
5	1-17-52	4+	-	-	-	K	60	0	0	0	0
6	"	2+	-	-	-	K	60	0	0	0	0
1	"	2+	4+	-	-	K	120	0	0	0	0
2	"	3+	2+	-	-	K	120	0	0	0	0
3	"	2+	2+	-	-	K	182	0	0	0	0
4	"	2+	2+	-	-	K	182	0	0	0	0
7	"	3+	2+	2+	-	K	244	0	0	0	0
8	"	2+	3+	2+	-	K	244	0	0	P	P
20	"	3+	3+	2+	-	DPn	296	0	0	0	0
19	"	3+	2+	2+	-	DPn	301	0	0	0	0
9	"	3+	2+	2+	-	K	305	0	0	0	0
10	"	2+	2+	2+	-	K	305	0	0	0	0
11	"	2+	2+	2+	-	K	358	0	0	0	0
12	"	4+	2+	2+	-	K	358	0	0	0	0
13	"	2+	2+	2+	2+	K	415	0	0	0	0
14	"	4+	3+	2+	2+	K	415	0	0	0	P
15	"	2+	2+	2+	1+	DPn	429	0	0	0	0
23	"	2+	2+	1+	1+	DPn	431	0	0	0	0
25	"	2+	4+	2+	1+	DPn	431	0	0	0	0
17	"	3+	2+	2+	1+	DPn	442	0	0	0	0
18	"	2+	2+	2+	2+	DPn	445	0	0	0	0
16	"	3+	2+	3+	3+	DPn	466	0	0	0	0
21	"	4+	4+	2+	2+	K	487	0	0	0	0
22	"	2+	3+	2+	2+	K	487	0	0	P	0
24	"	3+	2+	2+	2+	DPn	513	0	0	0	0

Key

Tuberculin Test:
- \- Not done
- 1+ Induration < 10 mm diameter
- 2+ Induration > 10 mm diameter
- 3+ Induration with ischemia
- 4+ Superficial ulceration

Tuberculosis:
- 0 No recognizable tubercles; no positive cultures
- NS Recognizable nonspreading tubercles
- IS Isolated spreading tubercles
- CO Isolated spreading tubercles with coalescence
- CA Extensive areas of coalescent tuberculous lesions with cavitation

- K Killed for sampling
- D Died
- Pn Nontuberculous pneumonia
- * No bacteriology
- P Positive

TABLE 6

INTRACUTANEOUS BCG VACCINATION (FRESHLY PREPARED VACCINE TICE STRAIN 924L) AND INHALATION OF QUARTZ DUST SIMULTANEOUSLY (Experiment 5 - #1189)

Guinea Pig Number	Date of Infection	Tuberculin Skin Test				Mode of Death	Time after Infection	Tuberculosis		Organisms Isolated	
		3-3-52	5-14-52	9-16-52	1-14-53			Lungs	Tracheo-bronchial Lymph Nodes	Lungs	Tracheo-bronchial Lymph Nodes
							days				
31	1-17-52	3+	-	-	-	K	60	0	0	0	0
32	"	3+	-	-	-	K	60	0	0	0	0
33	"	2+	2+	-	-	K	120	0	0	0	0
34	"	3+	2+	-	-	K	120	0	0	0	0
35	"	3+	4+	-	-	K	182	0	NS	0	0
36	"	3+	3+	-	-	K	182	0	0	0	0
49	"	2+	3+	-	-	DPn	201	0	0	0	0
37	"	3+	3+	2+	-	K	244	0	0	0	0
38	"	3+	3+	2+	-	K	244	0	0	0	0
39	"	3+	2+	2+	-	K	305	0	0	0	0
40	"	3+	2+	2+	-	K	305	0	0	0	0
41	"	3+	3+	4+	-	K	368	0	0	0	0
42	"	3+	2+	2+	-	K	368	0	0	0	0
55	"	3+	3+	3+	2+	DPn	419	0	0	0	0
43	"	3+	3+	2+	1+	K	425	0	0	0	0
44	"	3+	2+	2+	1+	K	425	0	0	0	0
50	"	2+	3+	2+	2+	K	464	0	0	0	0
45	"	4+	4+	2+	2+	K	487	0	0	0	0
46	"	3+	4+	2+	2+	K	487	0	0	0	0
47	"	3+	2+	2+	2+	K	547	0	0	0	0
48	"	2+	2+	2+	2+	K	547	0	0	0	0
51	"	2+	2+	2+	2+	K	547	0	0	0	0
52	"	3+	2+	2+	2+	K	547	0	0	0	0
53	"	3+	3+	2+	2+	K	547	0	0	0	0
54	"	2+	3+	2+	2+	K	547	0	0	0	0

Key: see TABLE 5

TABLE 7

INTRACUTANEOUS BCG VACCINATION (FRESHLY PREPARED VACCINE TICE STRAIN 924L)
INFECTION CONTROL ANIMALS
(Experiment 5 - #1189)

Guinea Pig Number	Date of Infection	Tuberculin Skin Test				Mode of Death	Time after Infection	Tuberculosis		Organisms Isolated	
		3-3-52	5-14-52	9-16-52	1-14-53			Lungs	Tracheo-bron-chial Lymph Nodes	Lungs	Tracheo-bron-chial Lymph Nodes
							days				
80	1-17-52	-	-	-	-	DPn	21	0	0	*	*
56	"	4+	-	-	-	K	60	0	0	0	0
57	"	3+	-	-	-	K	60	0	0	0	P
58	"	3+	4+	-	-	K	120	0	0	0	0
59	"	4+	4+	-	-	K	120	0	0	0	0
60	"	4+	4+	-	-	K	182	0	0	0	0
61	"	4+	4+	-	-	K	182	0	0	0	0
73	"	4+	4+	-	-	DPn	193	0	0	0	0
62	"	4+	4+	4+	-	K	244	0	0	0	0
63	"	4+	4+	3+	-	K	244	0	0	0	0
64	"	3+	3+	4+	-	K	305	0	0	0	0
65	"	4+	3+	3+	-	K	305	0	0	0	0
66	"	3+	3+	3+	-	K	368	0	0	0	0
67	"	3+	3+	3+	-	K	368	0	0	0	0
79	"	2+	3+	3+	3+	DPn	380	0	0	0	0
68	"	4+	4+	4+	3+	K	425	0	0	0	0
69	"	4+	4+	3+	3+	K	425	0	0	0	0
70	"	2+	4+	3+	3+	K	487	0	0	0	0
71	"	3+	3+	3+	2+	K	487	0	0	0	0
72	"	4+	4+	3+	3+	K	547	0	0	0	0
74	"	3+	3+	3+	4+	K	547	0	0	0	0
75	"	3+	3+	3+	3+	K	547	0	0	0	0
76	"	3+	3+	2+	2+	K	547	0	0	0	0
77	"	3+	4+	4+	3+	K	547	0	0	0	0
78	"	4+	4+	3+	3+	K	547	0	0	0	0

Key: see TABLE 5

TABLE 8

INHALATION OF QUARTZ DUST FOR 120 DAYS,
THEN INTRACUTANEOUS BCG VACCINATION (FOUR-DAY-OLD VACCINE TICE STRAIN 924L)
WITH INHALATION OF QUARTZ DUST CONTINUED
(Experiment 5-#1189)

Guinea Pig Number	Date of Infection	Tuberculin Skin Test				Mode of Death	Time after Infection	Tuberculosis		Organisms Isolated	
		3-3-52	5-14-52	9-16-52	1-14-53			Lungs	Tracheo-bronchial Lymph Nodes	Lungs	Tracheo-bronchial Lymph Nodes
							days				
81	9-17-51	3+	-	-	-	K	186	0	0	0	0
82	"	2+	-	-	-	K	186	0	0	0	0
83	"	3+	2+	-	-	K	186	0	0	0	0
84	"	2+	2+	-	-	K	186	0	0	0	0
85	"	2+	2+	-	-	K	308	0	0	0	0
86	"	2+	2+	-	-	K	308	0	0	0	0
87	"	4+	2+	2+	-	K	371	0	0	0	P
88	"	2+	3+	2+	-	K	371	0	0	0	0
93	"	4+	3+	2+	-	DPn	376	0	0	0	0
94	"	2+	2+	2+	-	DPn	385	0	0	0	0
91	"	3+	2+	2+	-	DPn	399	0	0	0	0
89	"	2+	3+	2+	-	K	431	0	0	0	P
90	"	3+	2+	2+	-	K	431	0	0	0	0
99	"	2+	2+	2+	-	DPn	483	0	0	0	0
92	"	2+	2+	2+	-	K	492	0	0	0	0
95	"	3+	2+	2+	-	K	492	0	0	0	0
100	"	3+	2+	2+	2+	DPn	497	0	0	0	0
96	"	2+	2+	1+	0	K	551	0	0	0	0
97	"	2+	2+	2+	1+	K	551	0	0	0	0
104	"	3+	2+	2+	2+	DPn	591	0	0	0	P
98	"	2+	2+	2+	2+	DPn	597	0	0	0	0
101	"	2+	2+	3+	2+	DPn	601	0	0	0	0
103	"	2+	2+	2+	0	DPn	609	0	0	0	0
102	"	3+	2+	2+	2+	K	612	0	0	0	0
105	"	2+	2+	2+	2+	K	612	0	0	0	0

Key: see TABLE 5

TABLE 9

INTRACUTANEOUS BCG VACCINATION (FOUR-DAY-OLD VACCINE TICE STRAIN 924L) AND INHALATION OF QUARTZ DUST SIMULTANEOUSLY
(Experiment 5-#1189)

Guinea Pig Number	Date of Infection	Tuberculin Skin Test				Mode of Death	Time after Infection	Tuberculosis		Organisms Isolated	
		3-3-52	5-14-52	9-16-52	1-14-53			Lungs	Tracheo-bronchial Lymph Nodes	Lungs	Tracheo-bronchial Lymph Nodes
							days				
106	1-21-52	3+	-	-	-	K	60	0	0	0	0
107	"	4+	-	-	-	K	60	0	0	0	0
108	"	3+	3+	-	-	K	121	0	0	0	0
109	"	2+	4+	-	-	K	121	0	0	0	0
110	"	2+	3+	-	-	K	182	0	0	0	0
111	"	3+	3+	-	-	K	182	0	0	0	0
112	"	3+	3+	2+	-	K	245	0	0	0	0
113	"	3+	3+	2+	-	K	245	0	0	0	0
128	"	2+	2+	2+	-	D	304	0	0	0	0
114	"	3+	3+	2+	-	K	305	0	0	0	0
115	"	4+	4+	2+	-	K	305	0	0	0	0
125	"	3+	3+	2+	-	DPn	308	0	0	0	0
116	"	3+	2+	2+	-	K	366	0	0	0	0
117	"	2+	3+	2+	-	K	366	0	0	0	0
118	"	3+	3+	2+	2+	K	424	0	0	0	0
119	"	4+	3+	2+	2+	K	424	0	0	0	0
130	"	4+	4+	2+	4+	DPn	441	0	0	0	0
124	"	2+	2+	2+	1+	K	486	0	0	0	0
129	"	3+	3+	3+	2+	K	486	0	0	0	0
123	"	3+	3+	2+	2+	DPn	539	0	0	0	0
120	"	3+	3+	2+	2+	K	547	0	0	0	0
121	"	3+	3+	2+	2+	K	547	0	0	0	0
122	"	3+	3+	2+	2+	K	587	0	0	0	0
126	"	3+	3+	2+	2+	K	547	0	0	0	0
127	"	3+	2+	2+	1+	K	547	0	0	0	0

Key: see TABLE 5

TABLE 10

INTRACUTANEOUS BCG VACCINATION (FOUR-DAY-OLD VACCINE TICE STRAIN 924L)
INFECTION CONTROL ANIMALS
(Experiment 5-#1189)

Guinea Pig Number	Date of Infection	Tuberculin Skin Test				Mode of Death	Time after Infection	Tuberculosis		Organisms Isolated	
		3-3-52	5-14-52	9-16-52	1-14-53			Lungs	Tracheo-bronchial Lymph Nodes	Lungs	Tracheo-bronchial Lymph Nodes
							days				
131	1-21-52	3+	-	-	-	K	60	0	0	0	0
132	"	3+	-	-	-	K	60	0	0	0	0
155	"	3+	-	-	-	DPn	63	0	0	0	0
133	"	3+	3+	-	-	K	121	0	0	0	0
134	"	4+	3+	-	-	K	121	0	0	0	0
135	"	4+	4+	-	-	K	182	0	0	0	0
136	"	3+	2+	-	-	K	182	0	0	0	0
137	"	4+	3+	3+	-	K	245	0	0	0	0
138	"	3+	3+	2+	-	K	245	0	0	0	0
139	"	3+	3+	3+	-	K	305	0	0	0	0
140	"	3+	3+	3+	-	K	305	0	0	0	0
141	"	3+	3+	3+	-	K	366	0	0	0	0
142	"	4+	3+	3+	-	K	366	0	0	0	0
143	"	3+	3+	3+	3+	K	424	0	0	0	0
144	"	3+	3+	4+	3+	K	424	0	0	0	0
145	"	4+	4+	3+	3+	K	486	0	0	0	0
146	"	3+	4+	3+	3+	K	486	0	0	0	0
147	"	2+	3+	4+	3+	K	547	0	0	0	0
148	"	4+	4+	3+	3+	K	547	0	0	0	0
149	"	3+	4+	3+	3+	K	547	0	0	0	0
150	"	4+	4+	3+	4+	K	547	0	0	0	0
151	"	3+	3+	3+	4+	K	547	0	0	0	0
152	"	3+	3+	2+	3+	K	547	0	0	0	0
153	"	3+	2+	3+	2+	K	547	0	0	0	0
154	"	2+	3+	2+	2+	K	547	0	0	0	0

Key: see TABLE 5

TABLE 11

SUMMARY OF DATA OF TABLES 5, 6, 7
INTRACUTANEOUS BCG VACCINATION (FRESHLY PREPARED VACCINE TICE STRAIN 924L)
(Experiment 5-#1189)

	Study I*	Study II**	Study III***
Duration of observations after infection	513 days	547 days	547 days
Number of guinea pigs observed	25	25	25
Number of guinea pigs with pulmonary tuberculosis	0	0	0
Number of guinea pigs with tuberculosis of the tracheobronchial lymph nodes	0	1	0
Number of guinea pigs with organisms isolated from the lungs	2	0	0
Number of guinea pigs with organisms isolated from the tracheobronchial lymph nodes	2	0	1

*Study I : Inhalation of quartz dust for 120 days, then intracutaneous BCG vaccination with inhalation of quartz dust continued.

**Study II : Intracutaneous BCG vaccination and inhalation of quartz dust simultaneously.

***Study III: Intracutaneous BCG vaccination; infection control animals.

TABLE 12

SUMMARY OF DATA OF TABLES 8, 9, 10
INTRACUTANEOUS BCG VACCINATION (FOUR-DAY-OLD VACCINE TICE STRAIN 924L)
(Experiment 5-#1189)

	Study IV*	Study V**	Study VI***
Duration of observations after infection	612 days	547 days	547 days
Number of guinea pigs observed	25	25	25
Number of guinea pigs with pulmonary tuberculosis	0	0	0
Number of guinea pigs with tuberculosis of the tracheobronchial lymph nodes	0	0	0
Number of guinea pigs with organisms isolated from the lungs	0	0	0
Number of guinea pigs with organisms isolated from the tracheobronchial lymph nodes	3	0	0

*Study IV: Inhalation of quartz dust for 120 days, then intracutaneous BCG vaccination with inhalation of quartz dust continued.

**Study V : Intracutaneous BCG vaccination and inhalation of quartz dust simultaneously.

***Study VI: Intracutaneous BCG vaccination; infection control animals.

TABLE 13

INTRACUTANEOUS BCG VACCINATION WITH FRESHLY-PREPARED VACCINE (TICE STRAIN 924L) IN DOGS WHOSE EXPOSURE TO QUARTZ DUST STARTED AT DIFFERENT PERIODS OF TIME AFTER VACCINATION (Experiment 7-#1200)

Dog Number	Vaccination			Dust Exposure		Mode of Death	Gross Pathology Lungs and Tracheobronchial Lymph Nodes
	Date of Vaccination	Site of Vaccination at 28 Days	Days Vaccinated	Date Started	Number of Days		
1*	3-24-52	Induration with ulceration	647+	0	0	*	
2*	"	"	647+	0	0	*	
3*	"	"	647+	3-25-53	647+	*	
4*	"	"	647+	10-15-52	647+	*	
5	"	"	17	3-25-52	17	D	Focal area of non-specific pneumonia.
6	"	"	540	3-25-52	540	K	Simple silicosis. No tuberculosis.

*Still living on December 31, 1953

TABLE 14

INTRACUTANEOUS BCG VACCINATION (TICE STRAIN 826L) AND DAILY SUBCUTANEOUS CORTISONE IN GUINEA PIGS
SKIN REACTIONS TO 0.1 CC. OF 3 PER CENT OLD TUBERCULIN
(Experiment 8-#1226)

	Number of Animals Exhibiting Each Type of Reaction						
Time after BCG vaccination, weeks	6	10	18	24	30	38	42
GROUP I Daily cortisone before and after vaccination	1+ - 0 2+ - 0 3+ - 12 4+ - 13	1+ - 0 2+ - 4 3+ - 17 4+ - 4	1+ - 0 2+ - 12 3+ - 9 4+ - 2	1+ - 1 2+ - 15 3+ - 4 4+ - 3	1+ - 4 2+ - 6 3+ - 9 4+ - 2	1+ - 1 2+ - 2 3+ - 12 4+ - 4	1+ - 0 2+ - 4 3+ - 9 4+ - 7
GROUP II Daily cortisone 3 months after vaccination	1+ - 0 2+ - 0 3+ - 16 4+ - 9	1+ - 0 2+ - 2 3+ - 13 4+ - 10	1+ - 0 2+ - 6 3+ - 11 4+ - 8	1+ - 5 2+ - 12 3+ - 4 4+ - 4	1+ - 1 2+ - 13 3+ - 8 4+ - 3	1+ - 2 2+ - 4 3+ - 10 4+ - 7	1+ - 0 2+ - 2 3+ - 9 4+ - 12
GROUP III Vaccination control No cortisone	1+ - 0 2+ - 0 3+ - 7 4+ - 12	1+ - 0 2+ - 2 3+ - 14 4+ - 9	1+ - 0 2+ - 3 3+ - 6 4+ - 14	1+ - 0 2+ - 6 3+ - 4 4+ - 13	1+ - 1 2+ - 10 3+ - 6 4+ - 6	1+ - 0 2+ - 3 3+ - 3 4+ - 15	1+ - 0 2+ - 0 3+ - 15 4+ - 16

Key

1+ Induration < 10 mm. diameter
2+ Induration > 10 mm. diameter
3+ Induration with ischemia
4+ Superficial ulceration